

**Studies on distribution patterns of
Ichthyopathogenic Bacteria
And their relation to the on set of
Disease in guppy rearing tanks**

2005

ABSTRACT

Management of the microbiological environment in tropical pet fish farms has become of utmost importance for exporters in the country. This is because bacterial diseases are fast becoming the main underlying cause in deteriorating quality of this highly exportable product.

The bacterial counts on the slime coating of fish are considered as a blueprint of the bacterial flora of their environment. Therefore, analysing the microbial population of water samples taken from culture tanks was considered as an indication of the possible bacterial diseases in fish. In this project attention has been focused on the bacterial diseases of fish in fresh water aquaria.

The main aim of this study is to identify the succession of bacteria in the disease causing mechanism and effect of growth of other types of bacteria present in water samples on the growth of *Flexibacter* spp. According to the Germ theory of disease, identification of the Ichthyopathogenic bacteria present in the water samples is another objective of this study.

Effect of physico chemical parameters of fish tank water on the growth of bacteria was also studied during this period.

Water samples were collected from five selected out growers who had the same variety of guppies (*Poecilia reticulata*) and age of fish in tanks during the same period of time. Dilution plate technique was used to enumerate bacteria from the collected water samples. Enumeration of heterotrophic bacteria was carried out using plate count agar and for Cytophaga species, Cytophaga agar (HSU shot medium) was used. Two replicate were carried out for each sample site.

Three types of recurring and significant colonies were identified from these samples. Colony counts were made and a statistical analysis on the different growth patterns of these types was carried out in order to trace a possible existence of a multiple correlation between them.

Morphological, physiological and biochemical characteristics of isolated bacteria were observed and identified. Pathogenic bacteria were investigated by isolation from water samples and experimental inoculation of isolated cultures to healthy fish. *Poecilia reticulata* was the selected species for the inoculating experiment. Samples were collected from the disease induced fish. Re-isolation and re-infection experiment was carried out again on healthy fish. Similarity of the symptoms and organisms were identified by the selected biochemical tests.

The gram positive bacteria isolated from water samples were *Bacillus subtilis* and *Streptococcus agalactiae*. *Flexibacter columnaris*, *Aeromonas hydrophila* and *Vibrio alginolyticus* were gram negative bacteria isolated from water samples. Two sub species of *Flexibacter columnaris* were identified from the water samples.

Multiple correlation analysis of the obtained data indicates that some gram positive bacteria (*Streptococcus agalactiae*, *Bacillus subtilis*) have a negative or a retarding effect on the growth of selected gram negative bacteria. (*Vibrio alginolyticus*, and

Aeromonas hydrophila). It was also indicated that the development and survival of *Flexibacter columnaris* in fish tanks is not affected by the presence of *Streptococcus agalactiae*, *Bacillus subtilis*, *Aeromonas hydrophila*, or *Vibrio alginolyticus*.

In the pathogenicity study, systemic infection developed in healthy *P. reticulata* after immersion in baths having isolates of *Flexibacter columnaris*, *Vibrio alginolyticus* and *Aeromonas hydrophila*. Other isolates *streptococcus agalactiae* and *Bacillus subtilis* did not induce lesions during infection experiment.

During the reisolation and reidentification experiment *Vibrio alginolyticus*, *Flexibacter columnaris*, *Plesiomonas shigelloids* and *Aeromonas hydrophila* identified as gram negative organism and *Streptococcus agalactiae* and *Bacillus megaterium* identified as gram positive organism.

From this pathogen *V. alginolyticus*, *F. columnaris* and *A. hydrophila* produces same symptoms in the reinfection experiment.

According to the Germ Theory of disease, *Flexibacter columnaris*, *Aeromonas hydrophila* and *Vibrio alginolyticus* are causative agents of body and fin rots, mouth rots, wounds and deep ulcers studied in this project. So they are considered as ichthyopathogenic organism.

During the study period pH, Ammonia, Nitrite values varies between these ranges, pH=7.1-8.7, NH₃= 0.1-0.5mg/l and NO₂⁻ = 0.5-0.1mg/l. During some weeks these values have exceeds the optimal ranges due to lack of water changes and has a bad effect on the physiology of fish.

Since the routine water changes were done in these water tanks could not be able to determine the exact effect of water quality on initiation of bacterial diseases. So that further studies should be carried out is recommended.